Simultaneous Quantitation of Hydrocodone and Metabolites Using UPLC-MS-MS

Erica Nachi ; Elizabeth Peterson; Brandon Retke; Chris Kafonek; Curtis Sheldon
Celerion, Lincoln, NE

OVERVIEW:

PURPOSE: To develop a rugged sensitive assay for the simultaneous quantitation of hydrocodone, hydromorphone, and norhydrocodone in human plasma by UPLC-MS-MS.

METHOD: Samples were extracted by a mixed-mode cation exchange solid phase extraction (SPE) procedure and analyzed by liquid chromatography/tandem mass spectrometry. An AB SCIEX API 4000™ was used to detect positive ions using electrospray ionization

RESULTS: Analytical challenges included imprecision and chromatographic interferences from the isobaric metabolites which were overcome in method development using UPLC chromatography on a Waters Acquity UPLC™ HSS T3 column. The method was successfully validated.

INTRODUCTION:

Hydrocodone (or dihydrocodeinone) is a semi-synthetic opioid derived from either of two naturally occurring opiates, codeine and thebaine. Hydrocodone is an orally active narcotic analgesic and antitussive (cough suppressant). It is commonly available in tablet, capsule, and syrup form, and is often compounded with other analgesics like paracetamol (acetaminophen) or ibuprofen. Hydrocodone is metabolized by CYP2D6 to form hydromorphone (active) and by CYP3A4 to form norhydrocodone (inactive). Sensitivity and selectivity challenges were successfully surmounted by chromatographic separation of the isobaric metabolites to accurately and precisely measure very low concentrations of hydromorphone (LLOQ 20 pg/mL). An UPLC-MS-MS was validated with an efficient runtime of 2.0 minutes.

Figure 1. Hydrocodone

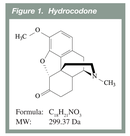


Figure 1. Hydromorphone

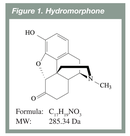


Figure 1. Norhydrocodone

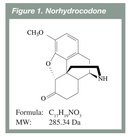


Figure 2. Post-column Matrix Infusion

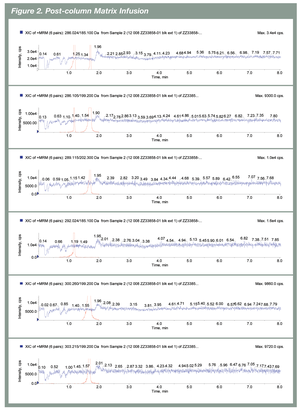


Figure 3. LLOQ Sample

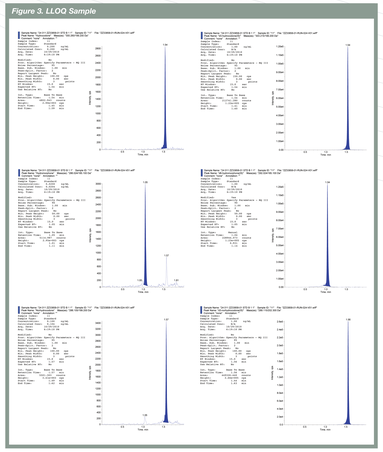
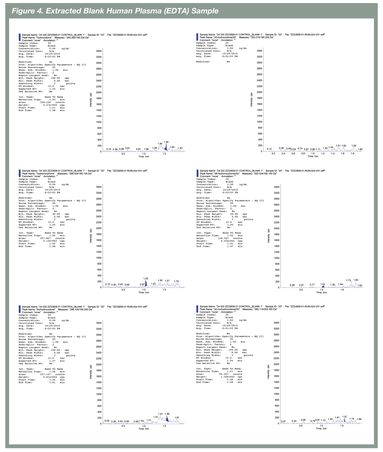


Figure 4. Extracted Blank Human Plasma (EDTA) Sample



METHODS:

- Aliquots (0.5 mL) of human plasma (EDTA) were spiked with deuterated internal standards (d_3-hydrocodone/ d_6-hydromorphone/ d_3-norhydrocodone) and extracted on a 96 well SPE plate (mixed mode cation exchange).
- The extracts were evaporated to dryness and reconstituted in ultrapure water for injection.
- Reversed phase gradient chromatographic conditions involved a Waters Acquity UPLC™ HSS T3, 50 x 2.1 mm, 1.8 µm column and mobile phases consisting of:
  - 0.1% HCOOH
  - 50:50:0.1 MeOH:H_2O:HCOOH
- The AB SCIEX API 4000™, using an ESI interface, was employed in this study.
- Positive ions were monitored in the MRM (multiple reaction monitoring) mode and data from the following MRM transitions:
  - 300.3 → 199.2 for hydrocodone
  - 303.2 → 199.2 for d_3-hydrocodone
  - 286.1 → 185.2 for hydromorphone
  - 292.1 → 185.0 for d_6-hydromorphone
  - 286.1 → 199.2 for norhydrocodone
  - 289.1 → 202.3 for d_3-norhydrocodone
- The acquisition time was 2.0 minutes.

RESULTS:

- Accurate and precise quantitation of LLOQ spiked samples in plasmas from different donors demonstrated reproducible sensitivity and lack of significant matrix effect in:
  - Hydrocodone 10 out of 10
  - Hydromorphone 9 out of 10
  - Norhydrocodone 9 out of 10
- Short-term stability in plasma was established for 25 hours at ambient temperature and in an ice water bath under white light.
- Freeze and thaw stability in plasma was established for six freeze (-20°C) and thaw (ambient and an ice water bath) cycles.
- Whole blood (EDTA) stability was established for 120 minutes in polypropylene tubes at ambient temperature under white light.
- Post preparative stability in injection solvent (quantitation against freshly extracted standards) was established for 180 hours at 5°C in a polypropylene 96 well plate.
- Processed sample integrity in injections solvent (re-injection stability) was established for 120 hours at 5°C in a polypropylene 96 well plate.
- Accurate quantitation of quality control samples spiked pre-extraction with the co-administered compound acetaminophen (25.0 µg/mL) was demonstrated.
- Selectivity was also successfully tested against Hydromorphone-3-ß-glucuronide (37.5 ng/mL). Long-term stability and freeze and thaw stability with Hydromorphone-3-ß-glucuronide were also tested at 46 days in polypropylene tubes at -20°C and 6 cycles.
- Extraction recovery tested across the entire linear range are as follows:
  - Hydrocodone 64% - 74% IS 67%
  - Hydromorphone 60% - 68% IS 62%
  - Norhydrocodone 48% - 53% IS 51%
- Hemolysis sample integrity was demonstrated with 5% whole blood in plasma at the LLOQ and high QC levels.
- Lipemic samples were evaluated and there was some difficulty passing them through the SPE sorbent. They will be evaluated on a case-by-case basis.

Table 1a. Matrix Effect for Hydrocodone in Human Plasma (EDTA)

		LLOQ		High	
Batch	Lot#	0.200 ng/mL	% Dev.	37.5 ng/mL	% Dev.
26	1	0.196	-2.0	36.6	-2.4
	2	0.217	+8.5	38.0	+1.3
	3	0.175	-12.5	36.3	-3.2
	4	0.188	-6.0	37.2	-0.8
	5	0.185	-7.5	35.2	-6.1
	6	0.190	-5.0	37.0	-1.3
	7	0.201	+0.5	36.8	-1.9
	8	0.202	+1.0	37.2	-0.8
	9	0.199	-0.5	35.7	-4.8
	10	0.200	+0.0	38.6	+2.9
Mean		0.195		36.9	
% CV		5.9		2.7	
% Theoretical		97.5		98.4	
n		10		10	

Table 1b. Matrix Effect for Hydromorphone in Human Plasma (EDTA)

		LLOQ		High	
Batch	Lot#	0.0200 ng/mL	% Dev.	37.5 ng/mL	% Dev.
26	1	0.0155	-22.5	3.83	+2.1
	2	0.0183	-8.5	3.99	+6.4
	3	0.0176	-12.0	4.03	+7.5
	4	0.0175	-12.5	3.70	-1.3
	5	0.0186	-7.0	3.63	-3.2
	6	0.0182	-9.0	3.73	-0.5
	7	0.0183	-8.5	3.80	+1.3
	8	0.0214	+7.0	3.91	+4.3
	9	0.0179	-10.5	3.71	-1.1
	10	0.0203	+1.5	3.88	+3.5
Mean		0.0184		3.82	
% CV		8.7		3.4	
% Theoretical		92.0		101.9	
n		10		10	

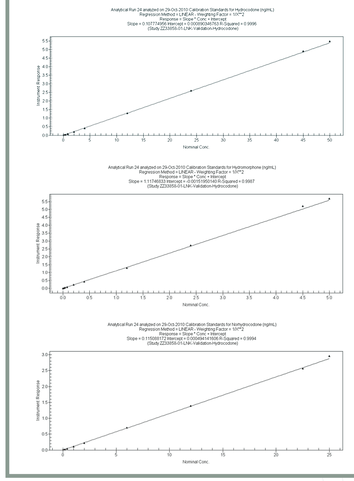
Table 1c. Matrix Effect for Norhydrocodone in Human Plasma (EDTA)

		LLOQ		High	
Batch	Lot#	0.100 ng/mL	% Dev.	18.8 ng/mL	% Dev.
26	1	0.0949	-5.1	18.5	-1.6
	2	0.101	+1.0	18.6	-1.1
	3	0.0747	-25.3	19.1	+1.6
	4	0.0939	-6.1	18.3	-2.7
	5	0.0843	-15.7	17.9	-4.8
	6	0.101	+1.0	18.4	-2.1
	7	0.0905	-9.5	18.4	-2.1
	8	0.0832	-16.8	18.4	-2.1
	9	0.106	+6.0	17.5	-6.9
	10	0.0966	-3.4	18.7	-0.5
Mean		0.0926		18.4	
% CV		10.3		2.4	
% Theoretical		92.6		97.9	
n		10		10	

Table 2. Precision and Accuracy for Quality Control Samples (LLOQ, Low, Mid, High)

Analyte	Analytical Range (ng/mL)	Intraday % Bias (< or =)	Intraday %C.V. (< or =)	Interday % Bias	Interday %C.V.
Hydrocodone	0.200-50.0	5.4%	1.4%	9.9%	5.0%
Hydromorphone	0.020-5.00	4.2%	6.0%	6.6%	1.5%
Norhydrocodone	0.100-25.0	5.9%	3.1%	7.7%	6.1%

Figure 5. Calibration Curves



CONCLUSION:

- A sensitive, rapid, selective, accurate and reproducible method for hydrocodone and metabolites was developed and validated.
- Method robustness was demonstrated with multiple column lots, mass spectrometers, and extraction scientists. Batch size validated was 192 samples.
- A post-column infusion demonstrated that hydrocodone and metabolites did not co-elute with any areas of significant suppression or enhancement.
- Highly effective and efficient chromatography was developed on an AB SCIEX API 4000™ that achieved lower limits of quantitation for hydromorphone of 20 pg/mL with an acquisition time of 2.0 minutes.

www.celerion.com